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# Effects of Chlordimeform on Cardiovascular Functional Parameters. Part 3. Comparison of Different Routes of Administration in the Postweanling Rat

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16. ABSTRACT

The effects of intraperitoneal (IP) administration of chlordimeform (CDM) were investigated in 22-24 day old pentobarbital-anesthetized Sprague-Dawley rats. Animals (N = 32) received a single IP injection of either 10, 30, or 60 mg/kg CDM, or normal saline vehicle. Heart rate (HR) and electrocardiogram (ECG) were monitored for all animals, while body temperature was maintained at control levels (37 °C). HR decreased 21-29% in the treated animals and there were no differences observed across dose groups or over time. ECG effects were confined to rate-related changes only. The decrease in HR produced by CDM in these animals was comparable to the delayed HR effect seen in previous studies from this laboratory (Watkinson, 1985; Watkinson, 1986) despite major differences in dosing regimens and routes of administration.

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# EFFECTS OF CHLORDIMEFORM ON CARDIOVASCULAR FUNCTIONAL PARAMETERS: PART 3. COMPARISON OF DIFFERENT ROUTES OF ADMINISTRATION IN THE POSTWEANLING RAT

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*The effects of intraperitoneal (ip) administration of chlordimeform (CDM) were investigated in 22- to 24-d-old pentobarbital-anesthetized Sprague-Dawley rats. Animals (n = 32) received a single ip injection of CDM at either 10, 30, or 60 mg/kg, or of normal saline vehicle. Heart rate (HR) and electrocardiogram (ECG) were monitored for all animals, while body temperature was maintained at control levels (37°C). HR decreased 21–29% in the treated animals, and there were no differences observed across dose groups or over time. ECG effects were confined to rate-related changes only. The decrease in HR produced by CDM in these animals was comparable to the delayed HR effect seen in previous studies from this laboratory, despite major differences in dosing regimens and routes of administration.*

## INTRODUCTION

The formamidine pesticide chlordimeform [*N'*-(4-chloro-*o*-tolyl)-*N,N*-dimethylformamidine, Galecron, Fundal, CDM] has been reported to produce a variety of biological effects in mammalian and insect animal models (Hayes, 1982). These reported effects have ranged from central nervous system stimulation and behavioral alterations (Gordon et al., 1985; Lund et al., 1979) to respiratory, renal, and cardiovascular complications (Lund et al., 1978a,b; Kimbrough, 1980). Despite the diversity of observed effects and proposed mechanisms of action, there is general agreement that the cardiovascular system constitutes a primary target organ for CDM toxicity.

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This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation.

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A series of studies from this laboratory (Watkinson et al., 1985; Watkinson, 1985, 1986) employed CDM as a prototypical toxic agent to demonstrate the applications of a recently developed electrocardiographic (ECG) analysis/screening procedure to cardiovascular toxicology. A most interesting finding of these studies concerned the biphasic nature of the toxic response observed following exposure to CDM. Briefly, intravenous administration of CDM produced acute, transient decreases in heart rate (HR) and blood pressure (BP) in conjunction with multiple arrhythmias and significant alterations in ECG waveform configuration. Following a period of 10–15 min of apparent recovery from the acute effects, there was a further abrupt and persistent decrease in HR and BP that was not accompanied by changes in ECG parameters. The dual character of the toxic response, in addition to representing an intriguing phenomenon, implicates a plurality of mechanistic actions.

The data presented in this report were obtained using an experimental protocol similar to those of the previous studies but that employed a different dosing regimen and route of administration for the toxic agent. The experimental design used was chosen for its simplicity and efficiency. Thus, this study was designed to permit comparisons with the results of the preceding studies in order to further clarify the nature of the toxic response to CDM as well as to illustrate the utility of the screening application of the previously described methodology (Watkinson et al., 1985).

## METHODS

### Animals

The animals used in the present study were obtained from the same source and treated in a similar manner to the animals used in the preceding study (Watkinson, 1986). Thus, young Sprague-Dawley rats were obtained (Charles River Breeding Laboratory, Kingston, N.Y.) at 21 d of age and housed in plastic cages on pine-shaving bedding in a temperature-controlled (22–26°C) and light-controlled (12 h light, 12 h dark) biologically clean room until studied. They were allowed access to tap water and laboratory feed ad libitum. Animals were of both sexes and ranged in age from 22 to 24 d old. The weight range for the female rats was 42–61 g, while the male rats ranged from 40 to 57 g.

### Experimental Preparation

The animals used in the present study were not surgically prepared with arterial and venous catheters like the animals in the preceding study (Watkinson, 1986). Otherwise, they were treated identically. Animals were anesthetized with sodium pentobarbital and placed on a heated water table (surface $T$ = 32°C). Electrodes were implanted sub-

cutaneously at the juncture of the extremities to monitor the ECG and thereby HR. All data were monitored on an eight-channel chart recorder and recorded on magnetic tape using a four-channel FM cassette recorder. ECG parameters were analyzed as previously described (Watkinson et al., 1985).

### Experimental Procedure

For this study, animals were paired by sex and weight and randomly assigned to one of four dosage groups. A total of 32 animals were used. Experiments were conducted in four blocks using eight animals at a time. There were two animals per group for each block; thus there was a total of eight animals per group for the study. Both ECG and HR were monitored continuously throughout the experimental procedure. Lead II was routinely monitored, with intermittent recordings of leads I and III obtained periodically. Preinjection control recordings were obtained after a period of 15–20 min of stabilized cardiovascular parameters following the initial preparatory procedures. Animals were then given a single injection of either 0, 10, 30, or 60 mg/kg CDM (chlordimeform HCl, 98% pure; Chem Service, Westchester, Pa.) dissolved in normal saline. All concentrations were adjusted so that the volumes administered were equivalent to 1 μl/g body weight. In addition to preinjection recordings, analysis of ECG parameters was conducted at 15, 30, and 60 min postinjection. All animals were monitored for at least 2 h following CDM exposure.

### Statistical Analysis

Statistical analysis was conducted using the general linear models procedure of the Statistical Analysis System (SAS Institute, 1982). The least square means procedure was used to test for differences between groups. Probability values less than 0.05 were considered significant.

## RESULTS

### Characteristic Response

Due to the nature of the ip injection, the characteristic biphasic response pattern seen in the previous studies employing iv administration was not observed. While it would not be appropriate to expect an acute response following ip exposure to CDM, the effects observed were similar to those observed previously during the delayed portion of the toxic response (Watkinson, 1985, 1986).

### Heart-Rate Response

The HR was monitored continuously for at least 20 min prior to ip injection of CDM and for at least 2 h postinjection. Although the values reported in Table 1 were taken at discrete time intervals, they are rep-

**TABLE 1.** Heart Rate and Electrocardiographic Waveform Parameter Values for Intraperitoneal Vehicle- and Chlordimeform-Treated Postweanling Rats

| Parameter | Preinjection, 0 min | Postinjection, 0 mg/kg | | |
|---|---|---|---|---|
| | | 15 min | 30 min | 60 min |
| HR (bpm) | 368.38 ± 19.91[b] | 379.48 ± 17.85 | 375.63 ± 10.95 | 382.39 ± 21.25 |
| P–T Interval (ms) | 165.77 ± 9.07 | 160.34 ± 7.58 | 163.48 ± 10.12 | 160.88 ± 11.01 |
| P–R Interval (ms) | 38.60 ± 1.31 | 38.38 ± 1.25 | 37.73 ± 1.04 | 38.70 ± 1.35 |
| R–αT Interval (ms) | 30.22 ± 1.43 | 28.25 ± 1.39 | 28.04 ± 0.83 | 27.69 ± 0.64 |
| R–T Interval (ms) | 127.17 ± 8.05 | 121.96 ± 6.78 | 125.75 ± 10.06 | 122.18 ± 10.11 |
| S–T Interval (ms) | 115.46 ± 7.66 | 111.15 ± 6.35 | 114.74 ± 9.46 | 111.42 ± 9.52 |
| P-Wave duration (ms) | 12.27 ± 0.67 | 11.54 ± 0.64 | 11.80 ± 0.52 | 11.79 ± 0.57 |
| QRS Duration (ms) | 15.10 ± 1.04 | 14.04 ± 0.81 | 13.75 ± 0.57 | 13.59 ± 0.56 |
| T-Wave duration (ms) | 112.07 ± 7.54 | 105.10 ± 6.79 | 103.39 ± 5.54 | 99.38 ± 4.50 |
| P–R Segment (ms) | 26.33 ± 0.72 | 26.84 ± 0.79 | 25.93 ± 0.73 | 26.91 ± 0.93 |
| S–T Segment (ms) | 3.38 ± 0.57 | 3.55 ± 0.57 | 3.17 ± 0.43 | 3.39 ± 0.49 |
| S–αT Segment (ms) | 18.50 ± 1.46 | 17.76 ± 1.56 | 17.48 ± 1.14 | 17.49 ± 0.99 |
| αT–P Segment (ms) | 96.95 ± 8.33 | 90.88 ± 7.63 | 89.08 ± 6.36 | 85.28 ± 5.11 |

| Parameter | Preinjection, 0 min | Postinjection, 10 mg/kg | | |
|---|---|---|---|---|
| | | 15 min | 30 min | 60 min |
| HR (bpm) | 393.37 ± 26.20 | 294.96 ± 15.12[d] | 292.59 ± 17.17[d] | 302.22 ± 18.73[d] |
| P–T Interval[a] (ms) | 157.97 ± 11.95 | 207.54 ± 10.92[c] | 209.78 ± 12.40[c] | 203.80 ± 12.85[c] |
| P–R Interval (ms) | 39.23 ± 1.84 | 40.04 ± 1.32 | 40.62 ± 1.38 | 40.21 ± 1.71 |
| R–αT Interval (ms) | 28.24 ± 1.27 | 27.05 ± 1.17 | 27.28 ± 1.12 | 26.97 ± 1.29 |
| R–T Interval (ms) | 118.74 ± 10.52 | 167.51 ± 10.40[c] | 169.15 ± 11.66[c] | 163.59 ± 11.96[c] |
| S–T Interval (ms) | 108.40 ± 10.31 | 156.58 ± 9.84[c] | 158.11 ± 11.08[c] | 153.04 ± 11.46[c] |
| P-Wave duration (ms) | 12.71 ± 0.87 | 13.75 ± 1.01 | 14.16 ± 1.29 | 13.75 ± 0.90 |
| QRS Duration (ms) | 14.37 ± 0.82 | 15.10 ± 0.88 | 15.11 ± 0.75 | 14.56 ± 0.76 |
| T-Wave duration (ms) | 104.37 ± 10.10 | 152.40 ± 9.55[c] | 154.04 ± 10.93[c] | 149.03 ± 11.27[c] |
| P–R Segment (ms) | 26.52 ± 1.23 | 26.29 ± 1.18 | 26.46 ± 1.11 | 26.46 ± 1.14 |
| S–T Segment (ms) | 4.03 ± 0.33 | 4.17 ± 0.32 | 4.06 ± 0.22 | 4.01 ± 0.24 |
| S–αT Segment (ms) | 17.80 ± 0.84 | 16.13 ± 0.86 | 16.09 ± 1.03 | 16.49 ± 1.00 |
| αT–P Segment (ms) | 91.00 ± 11.70 | 141.80 ± 11.07[c] | 144.13 ± 12.70[c] | 138.96 ± 12.91[c] |

| Parameter | Preinjection, 0 min | Postinjection, 30 mg/kg | | |
|---|---|---|---|---|
| | | 15 min | 30 min | 60 min |
| HR (bpm) | 377.40 ± 31.00 | 294.64 ± 21.42[d] | 291.53 ± 19.81[d] | 274.44 ± 34.02[d] |
| P–T Interval (ms) | 164.95 ± 13.11 | 210.45 ± 16.89[c] | 211.93 ± 16.02[c] | 268.66 ± 70.64[d] |
| P–R Interval (ms) | 38.86 ± 1.17 | 41.05 ± 1.75 | 42.09 ± 2.34 | 50.38 ± 10.36[d] |
| R–αT Interval (ms) | 27.42 ± 1.15 | 29.44 ± 3.41 | 29.30 ± 3.42 | 27.41 ± 1.44 |
| R–T Interval (ms) | 126.09 ± 12.44 | 170.91 ± 14.99[c] | 171.32 ± 13.74[c] | 220.81 ± 61.57[d] |
| S–T Interval (ms) | 116.13 ± 11.81 | 160.73 ± 14.23[c] | 160.99 ± 12.68[c] | 210.08 ± 60.65[d] |
| P-Wave duration (ms) | 12.22 ± 0.67 | 14.89 ± 1.78[c] | 15.57 ± 2.06[c] | 18.01 ± 3.31[d] |
| QRS Duration (ms) | 13.99 ± 0.60 | 14.29 ± 0.87 | 14.58 ± 1.36 | 14.73 ± 1.08 |
| T-Wave duration (ms) | 112.09 ± 11.91 | 156.62 ± 14.20[c] | 156.74 ± 12.45[c] | 206.08 ± 60.66[d] |
| P–R Segment (ms) | 27.09 ± 0.98 | 26.54 ± 1.48 | 27.07 ± 1.80 | 32.93 ± 6.68[c] |
| S–T Segment (ms) | 4.04 ± 0.29 | 4.10 ± 0.30 | 4.25 ± 0.42 | 4.00 ± 0.23 |
| S–αT Segment (ms) | 17.46 ± 1.54 | 19.26 ± 2.88 | 18.98 ± 2.63 | 16.68 ± 1.74 |
| αT–P Segment (ms) | 98.67 ± 12.94 | 141.47 ± 12.28[c] | 142.01 ± 10.83[c] | 193.40 ± 61.76[d] |

| Parameter | Preinjection, 0 min | Postinjection, 60 mg/kg | | |
|---|---|---|---|---|
| | | 15 min | 30 min | 60 min |
| HR (bpm) | 383.02 ± 27.28 | 267.16 ± 20.95[d] | 269.00 ± 18.44[d] | 282.63 ± 20.34[d] |
| P–T Interval (ms) | 162.67 ± 12.57 | 233.10 ± 15.83[d] | 229.36 ± 14.10[c] | 217.69 ± 14.40[c] |
| P–R Interval (ms) | 38.38 ± 1.68 | 40.72 ± 1.67 | 42.12 ± 1.67 | 41.05 ± 1.48 |
| R–αT Interval (ms) | 28.76 ± 1.37 | 33.38 ± 2.31[c] | 33.99 ± 2.91[c] | 33.95 ± 2.75[d] |
| R–T Interval (ms) | 124.30 ± 11.62 | 192.38 ± 14.45[d] | 187.23 ± 12.66[d] | 176.63 ± 13.08[c] |
| S–T Interval (ms) | 113.30 ± 11.71 | 180.49 ± 13.91[d] | 175.24 ± 12.15[d] | 164.27 ± 12.55[c] |
| P-Wave duration (ms) | 11.91 ± 1.09 | 13.86 ± 1.13[c] | 13.82 ± 1.09 | 13.18 ± 1.07 |
| QRS Duration (ms) | 14.47 ± 0.58 | 15.47 ± 0.77[c] | 15.55 ± 0.95[c] | 15.71 ± 1.12[c] |
| T-Wave duration (ms) | 109.83 ± 11.79 | 176.91 ± 13.89[d] | 171.68 ± 11.96[d] | 160.93 ± 12.24[c] |
| P–R Segment (ms) | 26.32 ± 0.91 | 26.86 ± 0.82 | 28.30 ± 0.98 | 27.87 ± 1.18 |
| S–T Segment (ms) | 3.47 ± 0.38 | 3.58 ± 0.35 | 3.56 ± 0.36 | 3.34 ± 0.53 |
| S–αT Segment (ms) | 17.76 ± 1.21 | 21.48 ± 1.83[c] | 21.99 ± 2.51[c] | 21.58 ± 2.31[c] |
| αT–P Segment (ms) | 95.54 ± 12.51 | 159.01 ± 12.51[c] | 153.25 ± 10.54[c] | 142.68 ± 11.51[c] |

[a] Definitions of electrocardiographic intervals, waves, and segments have been modified for the rat ECG. Although the terminology used is similar to traditional clinical usage, slight differences exist. For definition of terms, see Watkinson et al. (1985).

[b] Each value represents mean ± SEM for six to eight animals.

[c] Indicates significance at $p \leq 0.05$.

[d] Indicates significance at $p \leq 0.001$.

resentative of the complete data set and are most comparable to the delayed-response values in the previous study using iv administration (Watkinson, 1986). In addition, these data were normalized for each animal with respect to its preinjection control value, and these results are presented graphically in Fig. 1.

There was no difference in the preinjection HRs for any of the experimental groups. The HRs observed for the vehicle-treated group did not change over the course of the experiment. In contrast, the HRs of the animals in the three CDM-treated groups decreased significantly and remained depressed when compared to their preinjection control values or to the time-paired HRs of the vehicle-treated control group. The general trend indicated a relatively rapid decrease in HR, usually within 5 min, to a significantly lower level. These depressed HRs,

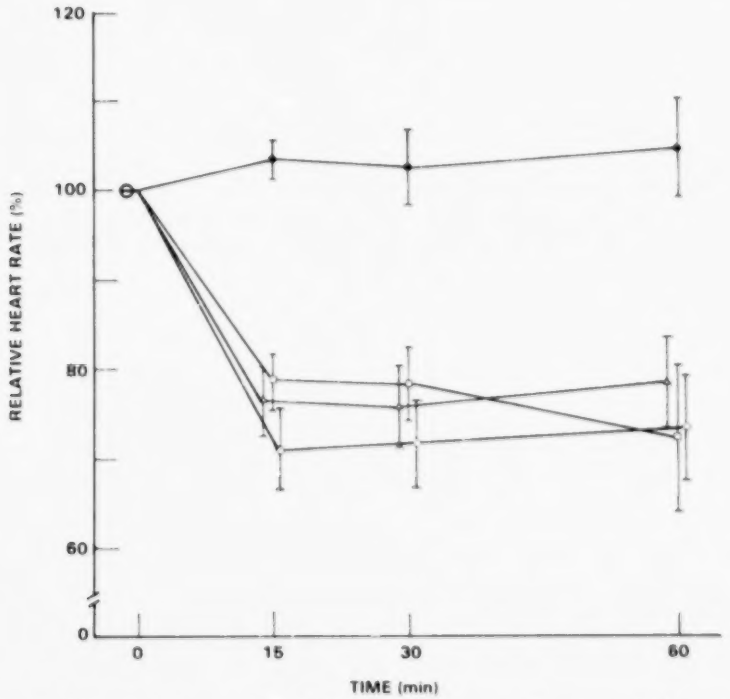


**FIGURE 1.** Relative HR response (mean ± SEM; $n = 8$) of postweanling rats to single intraperitoneal injection of CDM or normal saline vehicle. Data were first normalized with respect to preinjection values. Circle with bar (⊖) indicates initial control value for all groups. Dose groups are indicated as follows: ●, vehicle-injected; △, 10 mg CDM/kg; □, 30 mg CDM/kg; ◇, 60 mg CDM/kg. All postinjection values for the CDM-injected groups are significantly different from their preinjection control values ($p \leq 0.001$).

whose group means ranged from 71.0% to 78.7% of the original control values, were maintained throughout the duration of the experiment. There was no noticeable dose-response effect and no difference between the steady-state HR values of the three treatment groups following CDM exposure, an effect consistent with that seen for the delayed-response values in the preceding study.

### Electrocardiographic Response

**Waveform Parameters.** There were a number of significant changes in waveform parameters in this study, and these are shown in Table 1. However, as in the delayed-response data of the previous iv study, these changes are primarily rate-related. Specifically, these changes can be ascribed to a common factor, namely, an increase in the αT–P segment. The lengthening of the αT–P segment is an expected consequence of the decreased HR. Changes in non-rate-related parameters were minimal and include increases in P-wave and QRS-complex duration.

**Arrhythmias.** There were very few arrhythmias seen over the 2-h observation period. These arrhythmias were limited to occasional premature ventricular contractions and were seen in the higher dose groups. Again, these results are generally consistent with those obtained for the delayed-response data in the previous iv study. The primary exception to this statement involves those animals that subsequently died following CDM administration. In the period of time preceding death, these animals experienced a gradual decline in cardiovascular function. Often, generalized changes in P-wave amplitude and polarity as well as depression of the S–T segment area accompanied this declination in function.

### Lethality

There were 6 deaths among the 32 animals studied. Of these deaths, 3 occurred in the 30-mg/kg group and 3 occurred in the 60-mg/kg group. All of these deaths occurred during or just after the second hour of the 2-h observation period and were characterized by a gradual reduction in HR over a period of 5–10 min.

## DISCUSSION

An important purpose of the present study was to extend the observations of previous studies (Watkinson, 1985, 1986) with regard to the nature of the cardiovascular response following exposure to the pesticide CDM. This study was therefore designed to permit multiple comparisons with the results of earlier investigations. An additional, related purpose was to employ a modified experimental design and simplified experimental techniques to demonstrate the effectiveness of

the computer-assisted ECG analysis system (Watkinson et al., 1985) as a screening procedure for cardiovascular toxicology.

It was proposed (Watkinson, 1985) that the distinct response pattern involving both acute and delayed effects observed following iv exposure to CDM was attributable to separate mechanistic processes. The acute portion of the response was ascribed to a direct action of CDM on the musculature and conduction systems of the heart, while the delayed portion of the response was attributed to the action of a metabolite(s) of CDM on both the heart and peripheral vasculature. The results of the present study support the previous hypothesis.

Comparison of the cardiovascular and lethality data from the postweanling rats used in the present study with those from the postweanling rats in the preceding iv study (Watkinson, 1986) produced some unexpected but interesting correlations. The nature of ip exposure precludes an acute response; thus it is not possible to compare immediate postinjection data between the two groups of animals. However, appropriate comparisons can be made using the data from the steady-state or delayed portion of the response, after matching by dose and time. These calculations demonstrate remarkably similar changes in HR in both groups, despite major differences in dosing regimen (cumulative versus single injections) and route of administration (iv versus ip). For example, the values for the percent decrease in steady-state HR (delayed response) of the iv-group animals (Watkinson, 1986, Fig. 1) may be matched by dose and time with data from the ip group animals (present study, Fig. 1). Thus, 15 min after the 10-, 30-, and 60-mg/kg iv injections, the percent control HR values were 78.3, 75.7, and 71.7, respectively. The appropriate percent control HR values from the ip-group data at 15 min postinjection in the 10-, 30-, and 60-mg/kg groups were 76.3, 78.7, and 71.0, respectively.

It is also interesting to compare the HR effect following exposure to a single 60-mg/kg dose in both studies. The preinjection HR value for the ip group was 383 beats per minute (bpm). At 15 min following ip injection of CDM at 60 mg/kg, the HR had dropped to 267 bpm. The preinjection HR of the iv vehicle-treated control group was 436 bpm, a somewhat higher value that was most likely attributable to the more extensive surgical preparation involved. At 15 min following exposure to CDM, the HR decreased to 311 bpm. Thus, the average delayed HR decrease following ip exposure to 60 mg/kg was 29.0%, while iv injection of the same dosage caused a 28.7% drop in HR over the same time period.

The correlations between the results of these two studies are further strengthened by comparing the delayed HR response to CDM at 60 mg/kg under three different experimental conditions. These dissimilar conditions included prior cumulative iv doses of CDM, single iv doses of CDM, and single ip doses of CDM. Although the preinjection HR

values varied widely, the percent decreases in HR (28.3, 28.7, and 29.0, respectively) were extremely consistent. The effects of ECG parameters were also comparable during the delayed portion of the response.

The meaning of these observations, although very interesting, is unclear. The striking similarities between the data imply that similar mechanisms may be involved in the production of the delayed portion of the response. The consistency of the timing of the delayed effect and its relative independence from dosing regimen and route of administration support the hypothesis that a metabolite of CDM whose mechanism of action is saturated at relatively low concentrations may be involved.

The lethality data from the studies using postweanling animals provide additional evidence of potential differences in the mechanism(s) of the acute and delayed responses. All of the CDM-treated animals ($n = 15$) in the iv study died during the acute portion of the response. Thus, death occurred either during or immediately following injection and was typically characterized by abrupt cardiac arrest and a precipitous fall in BP to near-zero levels. In contrast, the deaths that occurred in the ip study ($n = 6$) were all during the delayed portion of the response. None of the animals died during the first hour following ip administration of CDM, and in all cases there was typically 5–10 min of steadily decreasing cardiac function prior to cardiac failure and death.

At the present time, none of the proposed mechanisms of action appear to adequately explain the observed effects. Although a major reported action of CDM involves inhibition of monoamine oxidase (Aziz and Knowles, 1973; Beeman and Matsumura, 1973) and monoamine oxidase inhibitors (MAOI) have been shown to lower blood pressure, it is unlikely that this mechanism is significantly involved in the production of the delayed response. Clinically, MAOI antihypertensives require several weeks to achieve significant reductions in BP (Nickerson and Ruedy, 1975).

CDM has also been linked symptomatically and mechanistically to local anesthetic agents, such as lidocaine (Chinn et al., 1976, 1977; Emran et al., 1980; Lund et al., 1978a,b), and it is possible that there may be a synergistic interaction with the general anesthetic sodium pentobarbital (Pfister et al., 1978). The effectiveness of this interaction may be challenged, however, on a pharmacokinetic basis. Specifically, in the present study, the concentration of sodium pentobarbital and the depth of anesthesia are greatest at the beginning of the experiment. The anesthetic is continuously being metabolized, a fact reflected by the gradual rise in HR observed in the vehicle-treated group over the duration of the experiment. The blood concentration of CDM, although not measured, probably peaks soon after ip injection. This statement is supported by the observation that the decrease in HR occurs within 15 min of injection and is stable thereafter. Thus, the

greatest potential for interaction—e.g., the time when maximal blood concentrations of both anesthetic and CDM are present—occurs between 5 and 15 min following ip injection of CDM. However, despite presumably decreasing blood concentrations of both agents, there was no attenuation of the delayed effects over the course of the experiment. Furthermore, all of the observed deaths in the ip study occurred at least 1 h following CDM injection, a time well past the predicted time of maximal interaction.

In summary, the present study provides further evidence for differential acute and delayed cardiovascular effects following CDM exposure in the rat. It is possible that these differences may be due to different mechanisms of action of CDM and/or a metabolite. This study also illustrates striking similarities between the time-delayed effects across age groups and experimental designs and thereby implicates the involvement of similar mechanisms of action in the production of the delayed effect. Finally, data are presented that illustrate the utility of the screening procedure previously described (Watkinson et al., 1985). By employing an experimental protocol similar to that described in the present study, it is a relatively simple procedure to obtain a dose-response profile of a potential toxic agent in a short period of time. Such studies may then be extended by using methodology similar to that described in the previous iv study (Watkinson, 1986).

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